

Pathogenesis of Glomerulosclerosis in Light Chain Deposition Disease

Role For Transforming Growth Factor- β

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The glomerulopathy of monoclonal immunoglobulin light chain deposition disease is a progressive disorder characterized by accumulation of monoclonal light chains and matrix proteins in the mesangium. To define the role of light chains in this process, cultured rat mesangial cells were exposed to different light chains and human albumin. Two light chains were purified from the urine of patients who had biopsy-proven light chain deposition disease. These proteins inhibited mesangial cell proliferation and increased production of matrix proteins, including type IV collagen, laminin, and fibronectin. By immunocytochemistry and bioassay, transforming growth factor- β (TGF- β) production and activity increased when mesangial cells were exposed to these proteins. Furthermore, anti-TGF- β antibody abolished the inhibition of cell proliferation and the increase of extracellular matrix protein production caused by these light chains. These findings were not observed in mesangial cells exposed to human albumin and two other light chains previously characterized to be tubulopathic. We concluded that the glomerulopathic light chains increased TGF- β , which inhibited mesangial cell proliferation and increased matrix protein production. Together with overexpression of TGF- β in affected glomeruli of light chain deposition disease, light chain-mediated stimulation of mesangial cells to produce TGF- β appears to be a key pathological mechanism of this disease. (Am J Pathol 1995, 147:375-385)

Renal failure is a prominent feature of the clinical course of monoclonal immunoglobulin light chain-related renal diseases. This complication develops in slightly more than half of patients with multiple myeloma and is second only to infection as the leading cause of death in these patients.¹ Renal failure can relate to glomerulopathy, proximal tubular damage, or cast nephropathy ("myeloma kidney"). Accumulation of monoclonal light chains in the glomerular mesangium and production of extracellular matrix proteins, which result in mesangial expansion and sclerosis, characterize the nodular glomerulosclerosis that occurs in light chain deposition disease.²⁻⁵ Progressive renal failure from this lesion is common and treatment has been unsatisfactory.^{2,3,5}

The mesangium is frequently involved in the course of glomerulopathy and serves as a target for a variety of mediators to enhance the glomerular injury.⁶ Because transforming growth factor- β (TGF- β) is involved in turnover and production of extracellular matrix proteins,^{7,8} the role of TGF- β in glomerulosclerosis has received considerable attention recently.⁹⁻¹¹ TGF- β stimulates matrix synthesis, inhibits matrix degradation, and stimulates synthesis of receptors for matrix proteins.⁷ Specifically, TGF- β increases production of type IV collagen, laminin, and fibronectin by glomerular cells.¹²⁻¹⁷ TGF- β can also act in either an autocrine or paracrine fashion to inhibit proliferation of glomerular mesangial cells.^{18,19}

Using immunoperoxidase staining, we recently found abnormal expression of TGF- β in nodular glomerulosclerosis associated with light chain deposition disease.²⁰ We hypothesized that certain immunoglobulin light chains stimulated mesangial cells directly to alter bio-

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logical function of these cells. To study this phenomenon, four monoclonal light chains from patients with either documented nodular glomerulosclerosis associated with light chain deposition or monoclonal light chain-related tubulopathy (cast nephropathy) were purified and incubated with rat mesangial cells in culture. Changes in growth, extracellular matrix expression, and TGF- β secretion by these cells were then examined. Development of a cell culture model may allow improved understanding of the pathophysiological events involved in this disease process and potentially provide novel treatment strategies to ameliorate or control the progression of this renal disease.

Materials and Methods

Protein Purification

Four human κ immunoglobulin light chains were purified from the urine in standard fashion using ammonium sulfate precipitation and ion exchange chromatography as described previously.²¹ Western blotting using goat polyclonal anti-human κ antibody (CAP-

PEL Research Products, Durham, NC) confirmed that the four light chains were κ isotypes. Glomerulopathic light chains (termed lin and gle) were obtained from two patients who had renal failure secondary to biopsy-proven monoclonal immunoglobulin κ light chain glomerulopathy (light chain deposition disease). These patients had classic nodular glomerular lesions (Figure 1) composed of extracellular matrix proteins and containing κ light chain. Typical of this lesion,²⁰ the mesangial nodules also contained TGF- β (Figure 1). Tubulopathic light chains (termed mic and rap) were obtained from two patients who had renal failure due to a tubulopathic effect by the light chains.^{22,23} Human serum albumin (alb) (Sigma Chemical Co., St. Louis, MO) was obtained commercially.

Rat Mesangial Cell Culture

Rat mesangial cell culture was performed using the standard method reported by Harper.²⁴ Briefly, kidneys were removed from 50-gm male Sprague-Dawley rats (Charles River Laboratories, Raleigh,

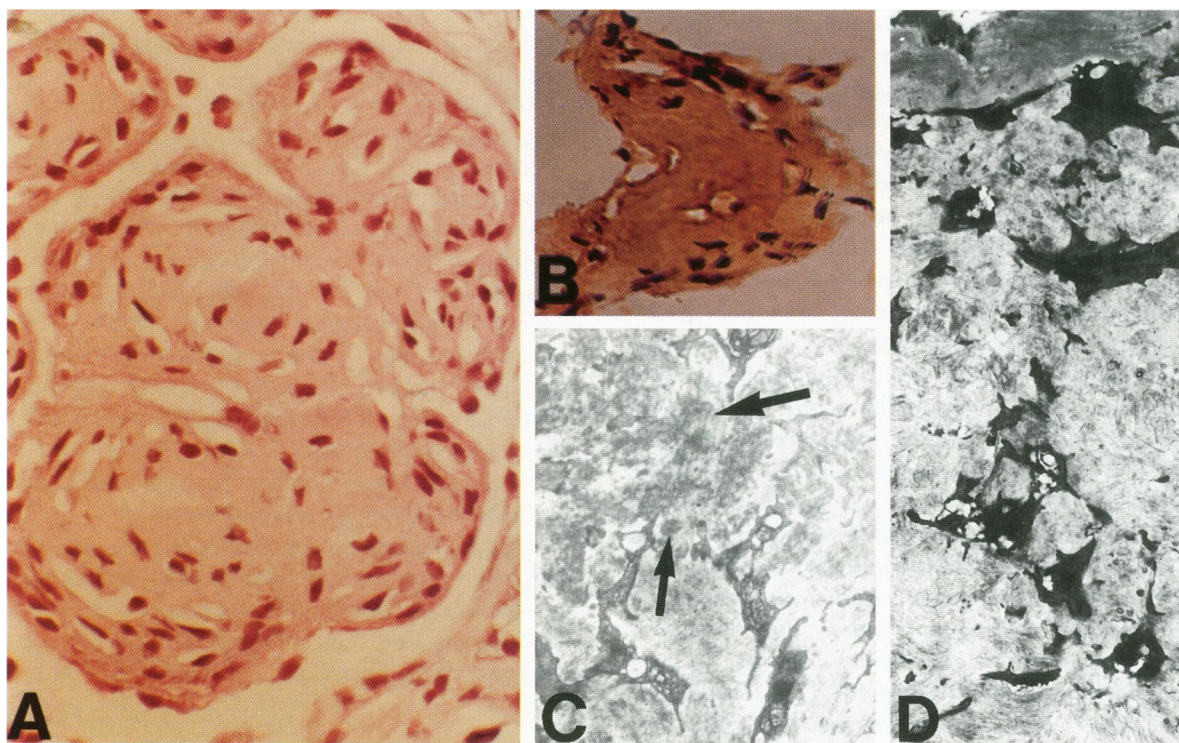


Figure 1. Light micrograph (A) of a glomerulus from a patient with monoclonal light chain deposition disease. The lesion was a classic nodular glomerulosclerosis. Expansion of the mesangial matrix with compression of the filtration surfaces of the glomeruli was observed. Hematoxylin and eosin, magnification $\times 500$. (B) Immunoperoxidase stain using rabbit polyclonal anti-human TGF- β antibody demonstrated diffuse strong staining of the glomerular mesangial nodules with TGF- β (single mesangial nodule illustrated). Avidin-biotin complex immunoperoxidase stain, magnification $\times 750$. Electron microscopic examination (C and D) documented classical findings. C illustrates an early phase of the mesangial expansion with abundant granular electron dense light chain material (arrows) surrounded by increased matrix. In D, the light chain material is virtually absent and the extracellular matrix, including fibrillar collagen, is strikingly increased (late phase). A spectrum of ultrastructural appearances, the extremes of which are shown, was present. Transmission electron microscopy, uranyl acetate and lead citrate stain. Magnifications: C, $\times 7500$; D, $\times 8000$.

NC). The cortex was separated and glomerular mesangial cells were isolated by sieving the cortex through stainless steel screens and a series of different pore size nylon sieves. The cells were plated on 100 \times 20 mm culture dishes (Becton Dickinson Labware, Lincoln Park, NJ) in RPMI 1640 (GIBCO Laboratories, Grand Island, NY) buffered with 12.5 mmol/L Hepes (Sigma Chemical Co.) at pH 7.4 and supplemented with 20% fetal calf serum (Hyclone Laboratories, Inc., Logan, UT), 100 U/ml penicillin (Sigma Chemical Co.) and 100 μ g/ml streptomycin (Sigma Chemical Co.). Once confluent, the cells were washed twice with Hanks balanced salt solution without Ca^{2+} and Mg^{2+} , then passaged after incubation with 5 ml of 0.25% trypsin and 0.01% EDTA (GIBCO Laboratories) for 15 minutes at 25°C. Cells in the first passage were used in this study. Ultrastructural examination and positive immunocytochemistry staining for muscle-specific actin and vimentin but absent keratin determined that these cells were a homogeneous population of mesangial cells.

Effect of Immunoglobulin Light Chains on Rat Mesangial Cell Function

Mesangial cells in their first subpassage were made quiescent by culturing them in medium containing 0.5% fetal calf serum for 3 days with a daily exchange of medium. Mesangial cells were then trypsinized, and 50 μ l of the cell suspension (1.5×10^6 cells/ml) were plated at a density of 75,000 cells/well into 96-well, flat-bottomed plastic microtiter plates (Costar Corporation, Cambridge, MA) and grown overnight. One application of lin, gle, mic, rap, and alb (1 to 10 μ g/ml) was administered into the medium of mesangial cells, which were observed subsequently for 1 to 4 days at 37°C, in 5% CO_2 . Cell proliferation was measured by a kit (Cell-Titer 96 Non-Radioactive Cell Proliferation Assay Kit, Promega Corporation, Madison, WI) that quantitated the reduction of a tetrazolium component into an insoluble formazan product by the mitochondria of viable cells. Proliferation of mesangial cells exposed to medium alone served as a control in each experiment. The results of experiments, which were each performed in triplicate, were calculated as percentage of control values and averaged. These studies were repeated three to six times. In addition, medium was collected to quantitate lactate dehydrogenase (LDH). LDH assay was performed using an autoanalyzer (COBAS FARA, Roche Diagnostic Systems Inc., Nutley, NJ) and a kit (LDH kit, Roche Diagnostic Systems Inc.).

This assay was based upon the catalysis by LDH of the reversible reaction between lactate and NAD to form pyruvate and NADH. NADH formation was used to quantitate LDH in solution.

To test the effect of the immunoglobulin light chains on accumulation of extracellular matrix protein, confluent mesangial cells grown on glass coverslips were exposed to lin, gle, mic, rap, and alb (10 μ g/ml) for 4 days. Immunoperoxidase staining was then performed using the avidin-biotin method modified from Matalon et al.²⁵ Mesangial cells on coverslips were rinsed three times in 20 mmol/L phosphate buffer, pH 7.5, and fixed with 100% methanol for 7 minutes at -20°C. After fixation, the coverslips were rinsed three times with phosphate buffer, then incubated in phosphate buffer containing 1.5% normal goat serum at 4°C for 10 minutes, followed by incubation with primary antibody, 1:100 dilution, for 35 minutes at 37°C. The primary antibodies used in this study included rabbit polyclonal anti-human fibronectin, rabbit polyclonal anti-human laminin, and rabbit polyclonal anti-human type IV collagen, all obtained from Chemicon International, Inc. (Temecula, CA). Negative controls were generated by replacing the primary antibody with nonspecific rabbit IgG or phosphate buffer. Unbound antibody was aspirated and the cells were rinsed three times with phosphate buffer. Coverslips were incubated with biotinylated goat anti-rabbit IgG (heavy and light chain-specific, Vector Laboratories, Burlingame, CA), 1:2000 dilution, for 30 minutes at 37°C in phosphate buffer containing 1.5% normal goat serum. Coverslips were rinsed three times with phosphate buffer and then incubated with avidin and biotinylated horseradish peroxidase (Vectastain Elite ABC Kit, Vector Laboratories) for 30 minutes at 25°C. After coverslips were rinsed three times with phosphate buffer, 0.1% diaminobenzidine tetrahydrochloride in 0.1 mol/L Tris buffer, pH 7.2, with 0.02% hydrogen peroxide was applied as the substrate of horseradish peroxidase for exactly 5 minutes at 25°C. Finally, coverslips were washed in tap water for 5 minutes, mounted with Immu-mount (Shandon Lipshaw, Pittsburgh, PA), and viewed with an Olympus microscope (Modulation Optics Inc., Greenvale, NY). To quantitate the intensity of the staining, 50 cells in each slide were evaluated in a blinded fashion at a 40 \times magnification with the intensity of staining of each cell determined to be: 0, no brown cytoplasmic staining identified; 1, weak, low intensity, generally focal, brown cytoplasmic staining (brown staining much weaker than background nuclear hematoxylin staining); 2, moderate, medium intensity diffuse brown cytoplasmic staining (background hematoxylin nuclear staining of about the same intensity as the brown cy-

toplasmic staining); or 3, marked, high intensity diffuse brown cytoplasmic staining (brown cytoplasmic staining overshadows background hematoxylin nuclear staining). The results for each slide were then added for a possible maximum staining score of 150. This experiment was repeated in triplicate and the scores were averaged. Extracellular staining for fibronectin, laminin, and collagen IV was also evaluated in areas where extracellular spaces were noticeable in between groups of three or more cells. Ten fields at 40 \times magnification were examined and each was graded and assigned a score according to the following criteria: 0, no brown extracellular staining identified; 1, weak, focal, low intensity brown extracellular staining noted; 2, moderate, medium intensity brown, extracellular staining (background nuclear staining as strong as extracellular staining); 3, marked, strong, high intensity brown extracellular staining (staining overshadowing nuclear staining). Determination of the degree of staining was performed blinded. Ten fields were added for a maximum total score of 30. The evaluation was repeated five times and the average estimated. The scores of intracellular and extracellular staining for fibronectin, laminin, and collagen IV were added for a possible maximum score of 180.

To evaluate further the effect of light chain on extracellular matrix protein production, mesangial cells were plated in six-well multiwell (35 mm) dishes (Corning, Corning, NY) until confluent and exposed to lin light chain (0 to 2 μ g/ml) for 4 days. After aspirating the media, the cells were rinsed three times with Hank's balanced salt solution with Ca^{2+} and Mg^{2+} . Solubilization of whole cell matrix proteins was performed according to the method published by Ayo and associates.²⁶ Protein concentration in each sample was quantitated by the bicinchoninic acid method.²⁷ Samples containing 100 ng total protein were loaded manually as a dot onto a nitrocellulose membrane. The membranes were then incubated in 5% nonfat dry milk in Tris buffered saline (TBS) followed by incubation with rabbit polyclonal anti-human fibronectin antibody (1:1000 dilution), rabbit polyclonal anti-human type IV collagen antibody (1:1000 dilution), and rabbit polyclonal anti-human laminin antibody (1:1000 dilution). After washing three times with TBS, goat anti-rabbit IgG conjugated with horseradish peroxidase (1:1000 dilution) (Bio-Rad, Richmond, CA) was added for 2 hours at room temperature, and the membranes were developed using peroxidase substrate (0.1 mmol/L Tris/HCl, pH 7.4, 0.8 mg/ml 3,3-diaminobenzidine, 0.01% hydrogen peroxide). Standards ranging from 1 to 100 ng were used

and applied in duplicate. The intensity of staining was determined using video densitometer (Model 620, Bio-Rad). A standard curve with its corresponding linear equation was created for each experiment. The specific measurements for each sample were derived from the standard curves. To investigate whether TGF- β was involved in the light chain-mediated increase of extracellular matrix protein production, confluent mesangial cells grown on glass coverslips were preincubated with rabbit anti-human TGF- β antibody (0 to 30 μ g/ml) or nonspecific rabbit IgG (0 to 30 μ g/ml) overnight; subsequently, the glc protein, 10 μ g/ml, was added and incubated for 4 days. Immunoperoxidase staining was then performed (as described above) to semiquantitate the production of extracellular matrix proteins, following the same grading scheme delineated above.

Effect of Immunoglobulin Light Chains on TGF- β Activity by Rat Mesangial Cells

Confluent mesangial cells were exposed to lin, mic, or alb (10 μ g/ml) for 1 to 4 days and medium was collected. TGF- β activity in harvested medium was assayed by determining colony formation of NRK cells (clone 49F, American Type Culture Collection, Rockville, MD) in soft agar. Briefly, NRK cell suspensions and TGF- β -containing samples were added in 0.3% agar with 5 ng epidermal growth factor in 10% calf serum on a base layer of 0.5% agar with 10% calf serum. The cells were incubated at 37 $^{\circ}\text{C}$ in 5% CO_2 for 7 days, and the number of colonies >62 μ m (>8 to 10 cells) in diameter was determined. Each sample was assayed in triplicate and averaged. The number of colonies correlated with TGF- β activity in solution and has been shown to be a reliable and reproducible measure of TGF- β activity.²⁸ In other studies, confluent mesangial cells grown on glass coverslips were exposed to lin, 10 μ g/ml, for 1 to 3 days. As described above, immunocytochemistry was used to semiquantitate the production of TGF- β using rabbit polyclonal anti-human TGF- β antibody (R&D Systems, Minneapolis, MN), 1:100 dilution. To investigate whether TGF- β was involved in the light chain-mediated inhibition of mesangial cell proliferation, quiescent mesangial cells were preincubated with rabbit anti-human TGF- β antibody (0 to 60 μ g/ml) overnight; then the lin protein, 10 μ g/ml, was added and incubated for 1 to 4 days. On each day, cell proliferation was determined and compared with controls that were incubated in medium alone.

Electron Microscopy/Ultrastructural Labeling

To study morphologically the interaction between mesangial cells and light chains, confluent mesangial cells in their first passage were exposed to 10 $\mu\text{g/ml}$ of lin and mic light chain and albumin for 4 days. Mesangial cells were then trypsinized and fixed in Carson-Millonig solution (Polysciences, Warrington, PA). The specimens were embedded in LR White resin (London Resin, Surrey, UK). Thick sections were prepared for survey. After selection of appropriate blocks, thin sections were cut and placed on 200 to 300-mesh, uncoated nickel grids. Grids were incubated with normal goat serum (Vector Laboratories), 1:30 dilution in 0.05 mol/L TBS, pH 7.6, for 30 minutes at room temperature followed by incubation with goat polyclonal anti-human κ light chain antibody (Cappel Research Products, Durham, NC), 1:100 dilution at 4°C overnight. The grids were washed in TBS for 20 minutes and then in TBS containing 1% bovine serum albumin for 10 minutes. Grids were incubated with gold-labeled rabbit anti-goat IgG serum (Jansen Pharmaceutica, Beerse, Belgium) 1:15 dilution in 0.02 mol/L TBS, pH 8.2, containing 1% BSA, for 1 hour at room temperature. After several washes using 0.05 mol/L Tris buffer, grids were post-stained with aqueous uranyl acetate and lead citrate (Fisher Scientific, Fairlawn, NJ).²⁹

Statistical Analysis

All data were expressed as mean \pm SEM. Comparisons among the groups were analyzed by analysis of variance and Fisher's protected least significant difference using statistical software (Statview, Abacus Concepts, Inc., Berkeley, CA). Statistical significance was set at the 5% level.

Results

Glomerulopathic Light Chains Inhibited Mesangial Cell Proliferation and Increased Extracellular Matrix Protein Production

Mesangial cell proliferation was inhibited in a time-dependent and concentration-dependent manner by the lin (Figure 2) and gle (data not shown) κ light chains from patients with monoclonal κ light chain glomerulopathy. In contrast, human albumin and tubulopathic κ light chains, mic (Figure 2) and rap (data

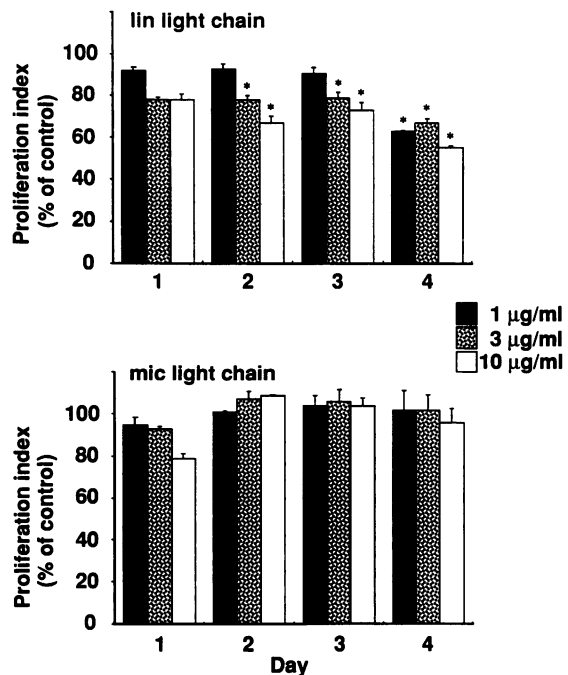


Figure 2. Effect of lin light chain ($n = 6$) and mic light chain ($n = 3$) on mesangial cell proliferation. Data from each experiment were standardized as a percent of control, which was represented by proliferation of cells exposed to medium alone. Exposure of cells to the lin light chain from 1 to 4 days in concentrations between 1 and 10 $\mu\text{g/ml}$ (top) decreased ($P < 0.05$) proliferation compared with cells exposed to the mic light chain (bottom) and alb (data not shown). The asterisk demonstrated different ($P < 0.05$) values among groups that used different concentrations on the same days of study. In comparing data from experiments that used the lin light chain, inhibition of mesangial cell proliferation was noted to occur in a time- and dose-dependent manner.

not shown), did not alter mesangial cell growth over the same time period. To demonstrate that growth inhibition was not due to an intrinsic toxicity of the protein, we used LDH release into the medium as a measure of cell viability. Incubation of mesangial cells with lin protein did not increase LDH concentration in the medium, compared with the corresponding samples that did not contain the light chain (Table 1). The LDH data confirmed trypan blue exclusion analysis of the mesangial cells and demonstrated the protein was not directly toxic. Analysis of immunoperoxidase

Table 1. LDH Concentrations in Medium of Mesangial Cells Cultured with the lin Light Chain for 1 to 4 Days

lin light chain ($\mu\text{g/ml}$)	LDH concentration (U/l)			
	24 hours	48 hours	72 hours	96 hours
0	63 \pm 1	78 \pm 6	94 \pm 4	105 \pm 16
1	63 \pm 3	78 \pm 6	85 \pm 1	96 \pm 14
3	57 \pm 3	78 \pm 9	75 \pm 3	88 \pm 0
10	62 \pm 1	68 \pm 1	75 \pm 1	91 \pm 4

staining demonstrated that the lin and gle proteins, 10 $\mu\text{g/ml}$, also dramatically stimulated production of type IV collagen, laminin, and fibronectin (Figure 3 and

Table 2). Increased production of these proteins was confirmed using a solid-phase dot blot immunoassay (Figure 4).

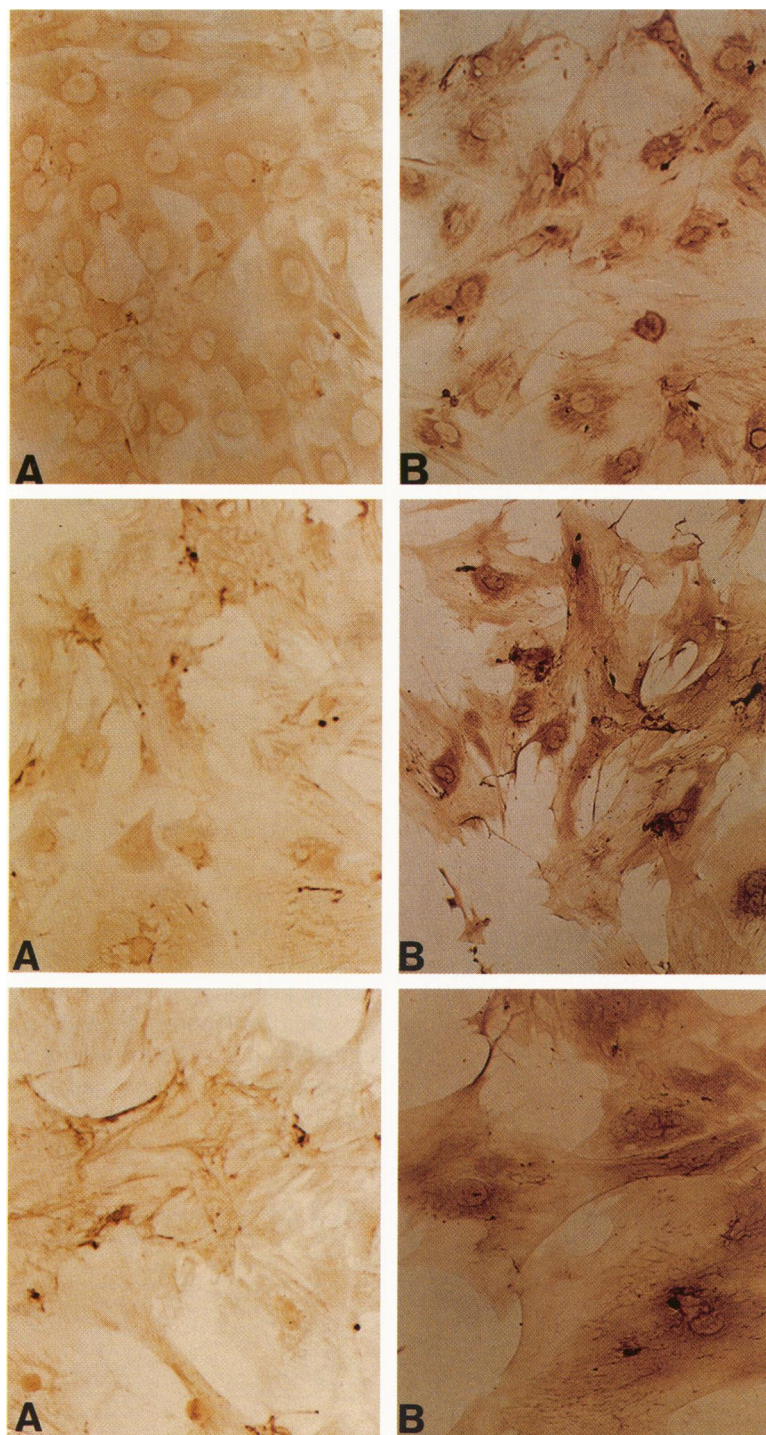


Figure 3. Immunoperoxidase staining of mesangial cells for fibronectin (top), laminin (middle), and type IV collagen (bottom). On the left (A) are representative cells cultured for 4 days and not exposed to light chain; on the right (B) are representative cells exposed for 4 days to lin light chain, 10 $\mu\text{g/ml}$. Staining for fibronectin, laminin, and type IV collagen was significantly increased in mesangial cells exposed to lin light chain. Avidin-biotin complex immunoperoxidase stain. Magnification: A and B $\times 250$, except for type IV collagen A and B ($\times 400$).

Table 2. *Stain Intensity Scores of Extracellular Matrix Proteins after Mesangial Cells Exposed to Test Proteins, 10 μ g/ml, for 4 Days*

Mesangial cells exposed to	Stain intensity scores		
	Type IV collagen	Laminin	Fibronectin
lin	130 \pm 9*	112 \pm 4*	113 \pm 5*
gle	134 \pm 2*	122 \pm 2*	125 \pm 2*
mic	65 \pm 4	62 \pm 3**	63 \pm 2
rap	57 \pm 1	55 \pm 2**	55 \pm 2
alb	58 \pm 3	41 \pm 2	51 \pm 2
Medium alone	51 \pm 2	48 \pm 3	46 \pm 5

* P < 0.05 compared with mic, rap, alb, or medium alone, n = 3.
 ** P < 0.05 compared with alb.

Glomerulopathic Light Chain Stimulated TGF- β Production

Exposure of mesangial cells to the lin protein increased TGF- β activity in the medium (Figure 5) and increased TGF- β staining determined by immunoperoxidase staining (Figures 6 and 7). In contrast, compared with medium alone, the mic protein and alb decreased TGF- β activity in the medium (Figure 5). To test whether the increase in TGF- β activity by the glomerulopathic light chains mediated the inhibition of mesangial cell growth and the increase of extracellular matrix protein production, mesangial cells were preincubated with different concentrations of anti-TGF- β antibody or nonspecific rabbit IgG for 24 hours preceding exposure to the light chain. The following day, lin protein or gle protein, 10 μ g/ml, was added and incubated for 1 to 4 days. The inhibitory effect of lin (Figure 8) and gle (data not shown) protein on mesangial cell proliferation and the increase of extracellular matrix protein production induced by gle protein (Figure 9) were reversed by addition of anti-TGF- β antibody. Anti-TGF- β antibody alone or nonspecific rabbit IgG had no direct effect on mesangial cell growth or matrix protein production.

Electron Microscopy/Ultrastructural Immunolabeling

Routine electron microscopic evaluation revealed prominent ruffling of cell membranes of mesangial cells exposed to lin protein, whereas no ruffling was noted in those cells exposed to alb or mic light chain. Immunoelectron microscopy using an anti- κ antibody demonstrated lin light chain in close association with the ruffled mesangial cell surfaces localizing on coated pit-like membrane areas (Figure 10). In contrast, no binding to the mesangial cell membrane was noted with the mic protein.

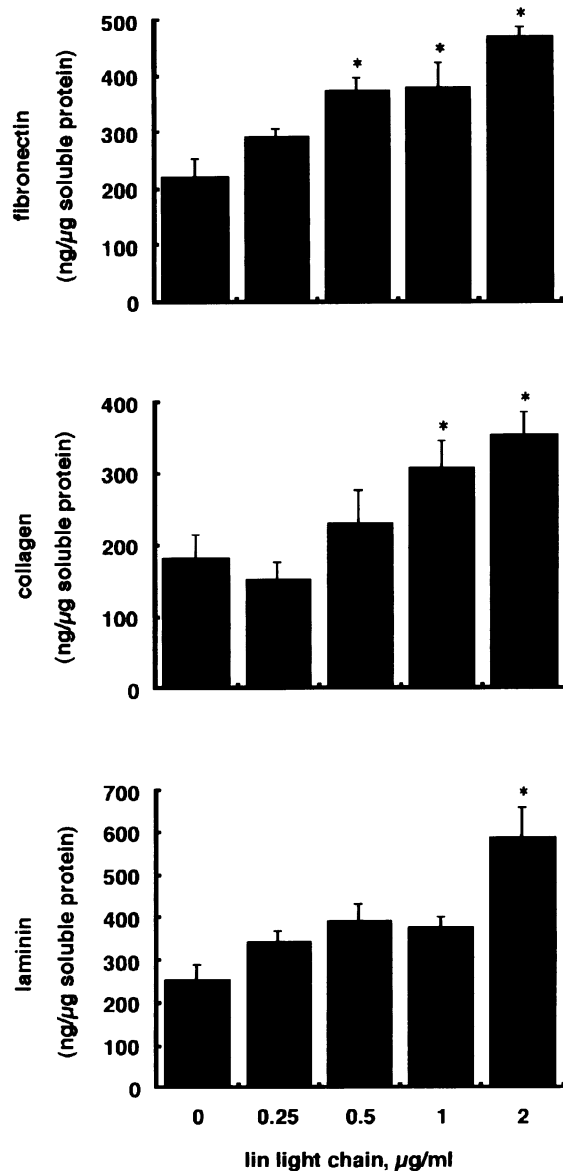


Figure 4. Dot blot immunoassay demonstrated the increase of fibronectin, laminin, and type IV collagen by cultured rat mesangial cells exposed to lin light chain (0 to 2 μ g/ml). The lin light chains increased matrix protein production from mesangial cells in a concentration-dependent fashion (n = 4). *Group that was different (P < 0.05) from the group of cells grown in medium alone.

Discussion

The glomerulopathy associated with monoclonal light chain deposition disease is characterized by the presence of granular deposits of light chains in the mesangium and subendothelial space.²⁻⁵ Extracellular matrix proteins accumulate in the mesangium to form nodules that compress the glomerular capillary filtering surfaces and ultimately produce global glomerulosclerosis, which is a predominant cause of end-stage renal failure in these patients.²⁻⁵ The

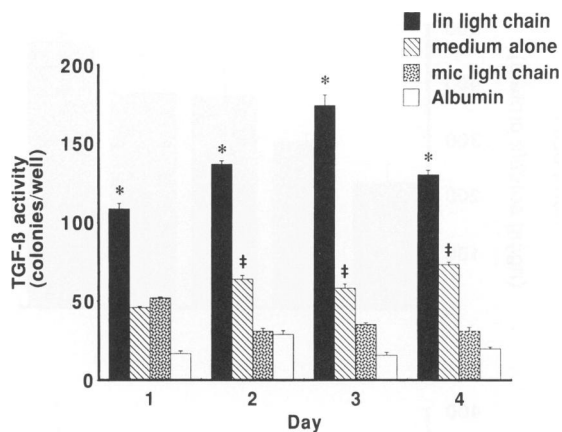


Figure 5. Effect of test proteins, 10 μ g/ml, on TGF- β activity, determined by bioassay, in medium of mesangial cells in culture ($n = 3$). In these experiments, the lin light chain increased ($P < 0.05$) TGF- β activity in the medium, compared with the other groups. TGF- β activity of mesangial cells exposed to medium alone was greater ($P < 0.05$) than that of cells exposed to the mic light chain and human alb. *Value greater ($P < 0.05$) than the other three groups of observations obtained on the same day of study. ‡Group that was greater ($P < 0.05$) than the values obtained using the mic light chain and alb. on the same day of study.

mechanism of excessive accumulation of extracellular matrix proteins was examined in our present study. Our data suggested that glomerulopathic light chains from two patients with light chain deposition disease bound to the mesangial cell membrane and stimulated these cells in culture to produce TGF- β , which inhibited cell growth and, as expected,^{7,12-17} increased extracellular matrix. These functional alterations were not seen when mesangial cells were incubated with tubulopathic light chains and albumin. Preincubation with anti-TGF- β antibody reversed cell growth inhibition and fibronectin production. Taken together with our previous results,²⁰ our current data support an important role for TGF- β in this disease process.

TGF- β is a 25-kd homodimer that is synthesized by numerous cell types including mesangial cells.^{7,19} This growth factor is known to be involved in regulation of cell proliferation^{7,18,19,30} and production of extracellular matrix proteins.¹²⁻¹⁷ TGF- β inhibits proliferation of glomerular mesangial cells^{18,19} and stimulates synthesis of collagen, fibronectin, and proteoglycans by mesangial cells in culture,^{9,12,15} causing accumulation of extracellular matrix. Introduction of the cDNA of the complete coding region of TGF- β into the rat kidney induces glomerulosclerosis.¹¹ The mechanisms of TGF- β -induced accumulation of extracellular matrix are not clear. However, TGF- β decreases the levels of collagenase, metalloproteinases, serine protease, and other enzymes that degrade matrix proteins.³¹ Cell proliferation is usually linked to the accumulation of extracellular matrix pro-

teins in the mesangium during the development of many renal diseases. Recently, these two processes have been dissociated. Eng et al³² reported that in rats with anti-Thy 1 glomerulonephritis subcutaneous injection of recombinant interferon- γ decreases mesangial cell proliferation, but does not reduce production of extracellular matrix proteins by mesangial cells. In addition, these investigators found that interferon- γ increases glomerular steady-state levels of mRNA of TGF- β .³² In our present study, we also found production of extracellular matrix proteins in the absence of cell proliferation. The precise mechanisms of the growth-inhibitory effect of TGF- β are uncertain. However, TGF- β antagonizes proliferation of epithelial cells stimulated by epidermal growth factor by decreasing the expression of *c-myc* gene.³³ TGF- β decreases transcription of *c-myc* by interacting with a *cis*-acting regulatory element in the *c-myc* promoter.³⁰ TGF- β also prevents phosphorylation of the protein product of the retinoblastoma gene (pRB), which is a negative growth regulator.³⁴

Overproduction of mesangial matrix along with deposition of light chains is the hallmark of light chain glomerulopathy and the apparent predominant cause of renal failure in these patients. Expression of TGF- β in affected glomeruli (Figure 1) is common.²⁰ Accumulation of extracellular matrix proteins in the mesangium is the central feature of many renal diseases including diabetic glomerulosclerosis. The renal lesions caused by monoclonal light chain deposition disease and diabetes mellitus are similar. Expansion of the mesangium and eventually glomerulosclerosis causes renal failure in both of these disease processes. In a diabetic animal model and during exposure of mesangial cells in culture to high glucose concentration, mesangial cell growth is increased,³⁵ collagen gene expression is stimulated,^{14,36} and extracellular matrix accumulation occurs.^{14,36} TGF- β has been proposed to play an important role in diabetic nephropathy. Elevated steady-state levels of TGF- β mRNA and expression of TGF- β protein were observed in glomeruli of diabetic patients and streptozotocin-induced diabetic rats³⁷ and in cultured mesangial cells exposed to high glucose.¹⁴

In summary, our present data suggest that certain light chains increased TGF- β activity, which stimulated secretion of extracellular matrix proteins and inhibited mesangial cell growth. The precise signaling mechanism of stimulation by light chains was not determined in this study. Light chains appear to possess specific physicochemical properties that confer renal pathogenicity.⁵ At this time, however, the property of the light chain molecule responsible for producing glomerular damage has not yet been identified, but is

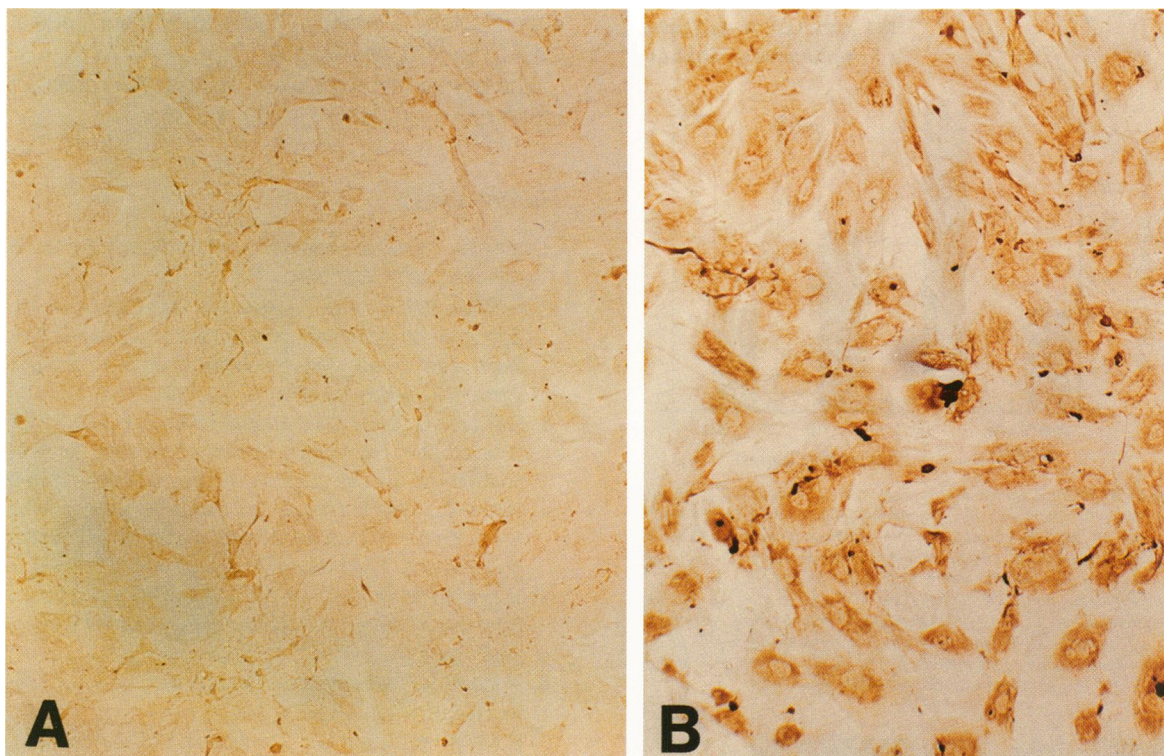


Figure 6. Immunoperoxidase staining, using anti-TGF- β antibody, of mesangial cells exposed for 4 days to either medium alone (A) or to lin light chain, 10 μ g/ml (B). TGF- β was obviously increased in mesangial cells exposed to lin light chain. Avidin-biotin complex immunoperoxidase stain. Magnification $\times 250$.

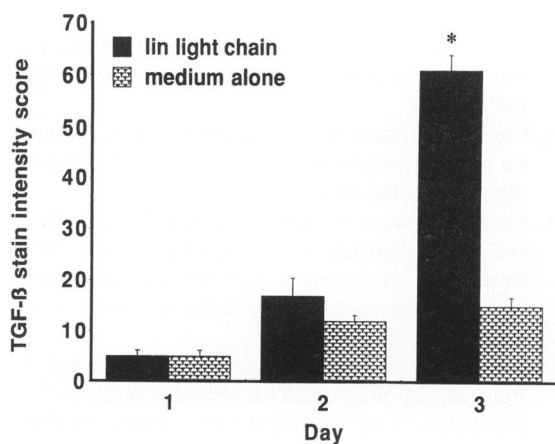


Figure 7. Quantitative data of immunoperoxidase staining, using anti-TGF- β antibody, of mesangial cells exposed to the lin light chain, 10 μ g/ml ($n = 3$ in each group). By the third day of study, the lin light chain increased ($P < 0.05$) TGF- β protein from mesangial cells, compared with TGF- β expression by cells grown in medium alone. * $P < 0.05$, compared with medium alone.

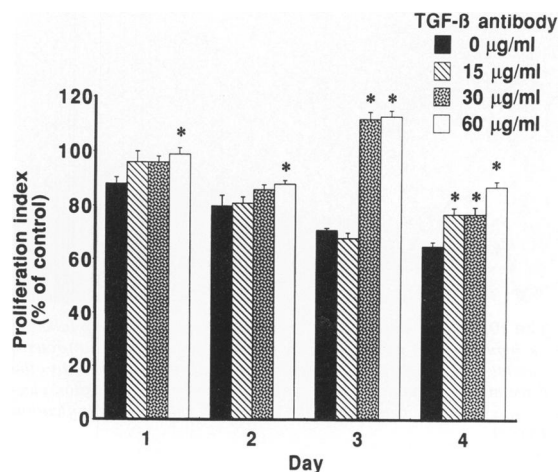


Figure 8. Effect of preincubation of mesangial cells with anti-TGF- β antibody, followed by exposure to the lin light chain, 10 μ g/ml ($n = 6$ in each group). Antibody was not added after the preincubation period. Data from each experiment were standardized as a percent of control (cells grown in medium alone). Anti-TGF- β antibody reversed the inhibition of mesangial cell proliferation induced by the lin light chain in a dose-dependent manner. *Group that was different ($P < 0.05$) from the group that did not contain anti-TGF- β antibody.

an active pursuit of this laboratory. Importantly, we produced a cell culture model of light chain-induced alteration of mesangial cell function. This model will be used to examine this process further. In addition, our cell culture model may potentially provide insight into new therapies that lower the morbidity and mor-

tality from renal failure in multiple myeloma. For example, decorin binds to TGF- β and neutralizes its biological activity.³⁸ Because decorin is a natural

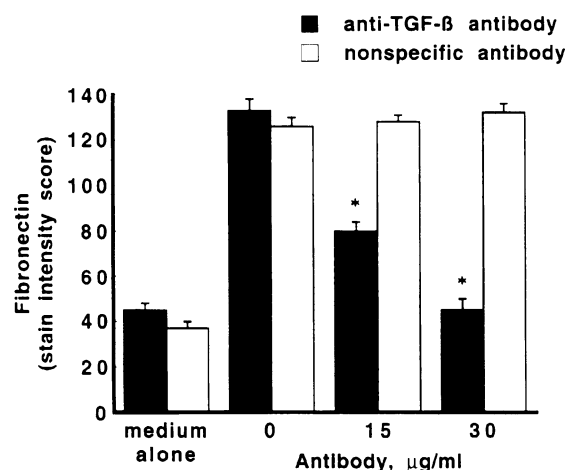


Figure 9. Preincubation of mesangial cells with anti-TGF- β antibody reversed the increase of extracellular matrix protein production caused by glc light chain in a dose-dependent manner. Mesangial cells grown on glass coverslips were preincubated with anti-TGF- β antibody or nonspecific rabbit IgG (0 to 30 $\mu\text{g/ml}$) overnight, followed by exposure to the glc light chain, 10 $\mu\text{g/ml}$, for 4 days. Immunoperoxidase staining of fibronectin was performed and quantitated ($n = 3$). Anti-TGF- β antibody, but not nonspecific rabbit IgG, reversed the increase of fibronectin production. *Group that was different ($P < 0.05$) from the nonspecific rabbit IgG group.

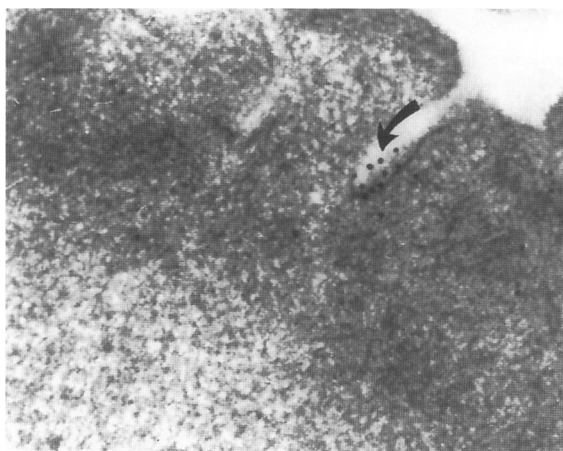


Figure 10. Immunoelectron (immunogold) microscopy with labeling for κ light chain of mesangial cells exposed for 4 days to lin protein, 10 $\mu\text{g/ml}$. Gold particles labeling κ light chain were localized to the cell membrane in coated pit-like areas as shown. Transmission electron microscopy, uranyl acetate and lead citrate. Magnification $\times 15,000$.

compound that accumulates in the kidney after intravenous administration,³⁸ use of this proteoglycan is a novel treatment that may prove efficacious in the management of the glomerulopathy of light chain deposition disease. Development of these concepts is a future direction of our laboratory.

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